Australian Standard®

Food microbiology

Method 28: Examination of specific products—Liquid milks and creams

PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Subcommittee FT-024-01, Food Microbiology to supersede AS 1766.3.13—1994.

The objective of this revision is to update the references and to transfer the procedure to AS 5013 series. It also incorporates minor technical variations on the apparatus used in the test technique.

METHOD

1 SCOPE

This Standard sets out microbiological methods for the examination of liquid milk and liquid milk products such as homogenized and modified milks, buttermilks and creams.

NOTE: The methods do not apply to cultured milk or cream products, or ultra-heat-treated (UHT) milks and creams.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

1166 Milk and milk products—Guidance on sampling

2300 Methods of chemical and physical testing for the dairying industry

2300.1.10 Method 1.10: General methods and principles—Determination of phosphatase activity

5013 Food microbiology (series)

5013.11.1 Method 11.1: Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—General rules for the preparation of the initial suspension and decimal dilutions

3 DILUENTS, CULTURE MEDIA AND REAGENTS

3.1 General

The diluents, culture media and reagents specified in this Clause shall be made up according to the formulations given in AS 5013 series.

3.2 Peptone salt solution

3.3 Trisodium citrate dihydrate ($Na_3C_3H_5O(COO)_3.2H_2O$) solution 2%

4 APPARATUS

The required apparatus is specified in the relevant methods in AS 1166, AS 5013 and AS 2300 referred to in this Standard.

5 SAMPLES

5.1 Laboratory samples

It is assumed that the laboratory sample has been obtained and delivered to the laboratory according to the procedures set out in AS 1166.

NOTE: The size of the laboratory sample taken in accordance with AS 1166 may be adjusted in order to provide a sufficient quantity for the required tests.

5.2 Storage and laboratory samples

Laboratory samples shall be stored at a temperature not exceeding 4°C, but without freezing, until tested. Testing shall commence within 24 h of sampling.

6 PREPARATION OF TEST SAMPLES

NOTE: For samples of pasteurized milk and cream to be prepared for phosphatase testing (see Clause 9), special instructions for preparation and mixing are given in AS 2300.1.10. These instructions should be followed in place of those given below.

6.1 Observation of physical condition and preparation for mixing

Observe the physical condition of the laboratory sample at the time of testing and record any abnormality. For cream samples only (except for phosphatase testing; see Note above), warm the sample to about 45°C, but not above. Proceed without delay to mixing (Clause 6.2) and the preparation of dilutions. The total time at 45°C shall not exceed 10 min.

6.2 Mixing of sample

6.2.1 *Liquid cream, liquid milks and liquid milk products*

Mix the sample well. If the first dilution is in a bottle, mix the contents by shaking 25 times in about 12 s through an arc of 30 cm. If the head space is insufficient for efficient mixing, aseptically pour the contents into a larger container for further mixing.

6.2.2 *Thick or coagulated cream*

Proceed directly to Clause 6.3.

6.3 Opening of containers

The procedure shall be as follows:

(a) Thoroughly clean the top of the container, treat with 70% ethanol (w/v) or 80% ethanol (v/v) and allow to dry.

(b) Open the closure aseptically, using scissors if necessary.

6.4 Preparation of dilutions for liquid milks and liquid milk products only

The procedure shall be as follows:

(a) Prepare dilutions of the sample as described in AS 5013.11.1, using peptone salt solution (3.2) or other diluent as specified in the AS 5013 series.

(b) Mix thoroughly and, using peptone salt solution (3.2), prepare tenfold dilutions as described in the AS 5013 series.

6.5 Preparation of dilutions for cream samples only

6.5.1 *Liquid cream*

The procedure shall be as follows:

(a) Weigh 10.0 ±0.1 g of sample.

(b) Dilute with 90 mL of peptone salt solution (3.2) prewarmed to about 45°C.

(c) Mix thoroughly and, using prewarmed peptone salt solution (3.2), prepare further tenfold dilutions as described in the AS 5013 series.

6.5.2 *Thickened or coagulated cream*

The procedure shall be as follows:

(a) Mix thoroughly by gently stirring with a spoon or spatula.

(b) Weigh 10.0 ±0.1 g of sample.

(c) Dilute with 90 mL of 2% trisodium citrate solution prewarmed to about 45°C.

(d) Mix thoroughly and, using prewarmed peptone salt solution (3.2), prepare further tenfold dilutions as described in the AS 5013 series.

7 GENERAL TESTS

7.1 Direct microscopic count

Proceed as described in Appendix A. If necessary, dilute the cream 1:10 with water of approved quality (see AS 5013) before preparing the smear.

7.2 Specific microorganisms

Use the relevant procedures described in AS 5013 to examine for specific groups of microorganisms.

NOTE: See Clause 8 below for thermoduric bacteria.

7.3 Test for penicillin

Use the disc assay method for penicillin*.

8 COUNT OF THERMODURIC BACTERIA IN RAW MILK AND CREAM

8.1 Laboratory pasteurization

Pasteurize the sample in accordance with the following procedure and at the same time use a pilot tube containing milk or cream in which a thermometer is immersed to monitor the temperature of the sample under test.

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* AS 1766.3.11—1991, *Food microbiology*, Method 3.11: *Examination of specific products—Dairy products—Test for penicillin.*

Completely immerse a screw-capped glass test tube or glass McCartney bottle holding 10 mL of sample, in water maintained at 63.5 ±0.5°C in a thermostatically controlled water bath fitted with a suitable agitator. The temperature of the sample shall reach 63°C within 5 min. After a further 30 min, remove the tube or bottle from the water bath and immediately cool to 5°C or below in iced water. In placing the container in the cooling water, avoid wetting the closure but ensure that the level of the water is above the level of the milk or cream. Upon removal from the iced water, wipe the tube or bottle with a tissue.

8.2 Colony count

Use the pour plate method described in AS 5013, using plate count agar and incubating at 30 ±1°C for 72 ±2 h.

9 PHOSPHATASE TEST FOR PASTEURIZED MILK AND CREAM

Carry out the phosphatase test in accordance with AS 2300.1.10, directly on the chilled, undiluted sample.

NOTE: After pasteurization, reactivation of phosphatase in cream can occur. Care should therefore be exercised in the interpretation of results.

10 TEST REPORT

The following information shall be reported:

(a) All details necessary for the complete identification of the sample.

(b) Reference to this Australian Standard, i.e. AS 5013.28.

(c) Date of testing.

(d) Any abnormality observed in the physical condition of the container or the product.

(e) Results of the tests.

(f) Any circumstance or conditions that may have influenced the results.

APPENDIX A

PROCEDURE FOR DIRECT MICROSCOPIC COUNT

(Normative)

A1 SCOPE

This Appendix sets out a method for counting the number of microbial cells and clumps in liquid milks and cultured milk products by microscopic examination of a stained film, viz. by a procedure not having a separate defatting step and which may be used on raw milk and similar products with low fat content in order to obtain results more rapidly.

NOTE: The degree of accuracy in estimating microbial numbers by this method depends on the number of microorganisms present in the milk. It is generally recognized that the degree of reproducibility by one operator decreases with counts of less than 500 000 per millilitre of milk, even when the technique is followed exactly.

Factors contributing to this poor reproducibility within and between operators are inaccuracies in measuring volumes, area of smear, inadequate fixing and staining, poor microscopy, irregular distribution of microorganisms, small number of fields actually counted in proportion to fields present in the smear, and eye fatigue.

A2 DEFINITIONS

For the purpose of this Standard, the definitions below apply.

A2.1 Microbial clump count

Microbial count based on groups of microorganisms which appear to be the same type: cell of like kind within 5 μm of a group are deemed to belong to that group, regardless of closeness to each other, cells of different types are deemed to be separate clumps.

A2.2 Individual count

Microbial count of all individual cells, both within groups and in isolation.

A3 REAGENTS

A3.1 Methylene blue staining solution (modification of Newman Lampert Stain)

Add, gradually, 0.5 g of methylene blue chloride (certified grade) to a mixture of 56 mL 95 percent ethanol and 40 mL xylene (technical grade) in a stoppered 200 mL flask. Dissolve by swirling the flask. Stand overnight (12 h to 24 h) under refrigeration (0°C to 4°C). Filter through fine paper* and add 4 mL glacial acetic acid to filtrate. Store in a tightly closed bottle in a cool dark place.

WARNING: PREPARE THE STAIN UNDER AN EXHAUST HOOD TO AVOID INHALATION OF TOXIC SOLVENT FUMES.

NOTE: As this dye has limited shelf life, discard the solution if it becomes contaminated or shows signs of deterioration.

A4 APPARATUS

A4.1 Pipette or micropipettor

Calibrated to deliver 0.01 mL.

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* Whatman No. 42 or equivalent has been found to be satisfactory.

A4.2 Microscope

Provided with an oil immersion objective, with field diameter in the range 146 μm to 206 μm.

A4.3 Microscope slides

Clean, unscratched, and treated to remove grease.

A4.4 Guide plate (template)

To outline an area of 1 cm^2 when positioned underneath a microscope slide.

A4.5 Suitable 'rake'

A5 PREPARATION OF SAMPLE

The milk/cultured dairy products shall be mixed before testing by shaking the container 25 times in about 12 s through an arc of 30 cm. If the head space is insufficient for efficient mixing, aseptically pour the contents into a larger sterile container for further mixing.

A6 METHOD WITHOUT DEFATTING

WARNING: CARRY OUT THE PROCEDURE IN A FUME CUPBOARD TO AVOID INHALATION OF TOXIC SOLVENT FUMES.

A6.1 Preparation of smear for microscopic examination

The procedure shall be as follows:

(a) Thoroughly rinse the pipette or micropipettor with the mixed sample and discharge to waste.

(b) Deliver the 0.01 mL of sample onto a clean microscope slide, making sure that the surface of the slide is horizontal. Spread immediately over the outlined area of 1 cm^2, using a cool rake.

(c) Dry the film at 40°C to 45°C in a dust-free atmosphere, in a horizontal position.

A6.2 Staining

The procedure shall be as follows:

(a) Flood the slide with the methylene blue staining solution (see Paragraph A3.1) and leave for 2 min.

(b) Drain the slide of excess solution by resting the edge on absorbent paper.

(c) Dry the slide, using a warm air draught if available.

(d) Rinse the slide by dipping into three successive lots of warm tap water at 35°C to 45°C. Drain and air dry.

NOTE: Fresh rinse water should be used for each slide.

A7 MICROSCOPIC EXAMINATION OF STAINED SMEARS

A7.1 Mounting the slide

The slide shall be mounted in the microscope (see Paragraph A4.2).

A7.2 Number of fields to be counted

The number of fields to be counted shall be determined by conducting a preliminary microscopic survey of the mounted smear. Table A1 specifies the number of fields to be counted according to the number of organisms per field.

TABLE A1

NUMBER OF FIELDS TO BE COUNTED FROM SMEAR

Average number of organisms per field	Number of fields to be counted
0–3	64
4–6	32
7–12	16
13–25	8
26–50	4
51–100	2
Over 100	1

A7.3 Selection of fields

A7.3.1 *General*

Having determined the number of fields to be counted (see Paragraph A7.2), select fields at approximately equidistant intervals over the smear. Do not consciously select the field to be counted while looking down the microscope as this may introduce a subjective bias.

A7.3.2 *One field*

Select at about the centre of the smear.

A7.3.3 *Two fields*

Select the fields at the quarter distances from the ends of one of the diagonal lines across the smear.

A7.3.4 *Four fields*

Select the fields at four points midway between the centre and the four corners.

A7.3.5 *Eight fields*

Select three rows of two or three fields at equidistant intervals between the north and south sides.

A7.4 Counting

For each selected field, the number of microbial clumps and number of microbial cells shall be counted.

A8 CALCULATIONS

A8.1 Microscope field area

The area of the field (mm^2) is determined as follows:

(a) Place a stage micrometer under the microscope, and using the oil immersion lens, determine the diameter of the field. The field diameter should be in the range 0.146 to 0.206 (146 μm to 206 μm).

(b) Calculate the area of one field using the formula Area = 3.14 R^2 (where R is the radius, or half the diameter, in mm).

(c) Recalculate the field area after serving of the microscope or if the lens system is changed.

A8.2 Microscope factor (MF)

The microscope factor is the reciprocal of the actual fraction of sample seen in one microscope field, and is expressed to two significant figures. The microscope factor is calculated from the following equation:

$$MF = \frac{\text{Area of smear, in square millimetres}}{\text{Microsope field area, in square millimetres}} \quad \ldots \text{A8(2)}$$

A8.3 Microscopic count

Calculate the average number of microbial clumps and the average number of microbial cells per field. Using the microscope factor, in conjunction with the averages obtained and sample volume, calculate the number of clumps and of individual bacteria per millilitre from the following equation:

$$\text{Microscopic count per mL} = \frac{MF \times \text{Average number per field}}{\text{Volume of sample, in millilitres}} \quad \ldots \text{A8(3)}$$

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